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Development of a novel biochar-made porous monolith for enhanced C1 and H_2 fermentation ☆

Yusuf Küçükağa [a,b,c,*], Andrea Facchin [a,b], Aaron Alfonsi [b], Federica Costantini [b,d], Serdar Kara [c], Cristian Torri [a,b]

[a] Department of Chemistry "Giacomo Ciamician", University of Bologna, Laboratori "R. Sartori", Via Sant'Alberto 163, Ravenna 48123, Italy
[b] Interdepartmental Research Center for Research in Environmental Sciences (CIRSA), University of Bologna, Via Sant'Alberto, 163, Ravenna, Italy
[c] Environmental Engineering Department, Faculty of Engineering, Gebze Technical University, Kocaeli 41400, Turkiye
[d] Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, Italy

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ABSTRACT

Biochar is a carbonaceous porous material that is produced through the thermal processing of biomass under oxygen-limited environment. Nevertheless, biochar is known to be an inexpensive and sustainable raw material with a wide range of possible applications. Recently, biochar has been discovered as an efficient biological catalyst for anaerobic conversion, mainly due to its highly porous structure with micro and macro channels, which procures a viable living area for attached-grown microorganisms. Whereas it is never applied to improve the biological conversion of gas substances such as C1 (e.g., CO, CO_2) and H_2, which is a promising research area with increasing commercial interest. However, considering that biological reaction is limited by the target water solubility of gas substrates, special attention is required when combining biochar for gas fermentation. The goal was to create a novel gas sparger where the biofilm grows on biochar, thus improving the interaction with the gaseous substrate. For this purpose, polystyrene foam and powdered biochar were compounded to form a mouldable composite, which was then cast as a porous monolith.

- Biochar-made sparger (BS) was investigated for the homoacetogenic conversion of H_2 gas via microbial mixed cultures as opposed to a control test equipped with a stone sparger.
- BS showed a significantly better performance in terms of biological gas fixation rate (36% more than control) and productivity (8.5 g_{COD} L^{-1} d^{-1}).

☆ Related research article Y. Küçükağa, A. Facchin, V. Stefanelli, F. Costantini, S. Kara, C. Torri, Innovative Char-based sparger for Improving Volatile Fatty Acids (VFA) Production in Homoacetogenic Fermentation of H2/CO2 with Microbial Mixed Cultures (MMC) Chem. Eng. J. https://doi.org/10.1016/j.mex.2023.102296 (Published: 23 July 2023).

* Corresponding author at: Department of Chemistry "Giacomo Ciamician, "University of Bologna, Laboratori "R. Sartori", Via Sant'Alberto 163, Ravenna 48123, Italy.
E-mail addresses: yusuf.kucukaga@unibo.it, yusufkucukaga@gmail.com (Y. Küçükağa).

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Method details

Background

Biochar obtainment from biomass sources has a widely applied ancient process. Biochar is a highly porous and usually a conductive material that is obtained by applying heat to biomass under oxygen-limited environments. In recent years, biochar has been found to be capable of enhancing biological processes, such as water treatment, composting and biofiltration [1–3]. It has also been proven to be a promising microbial environment for several anaerobic processes [4–7], particularly for acidogenic fermentation [8,9]. In this regard, the use of biochar as a microbial enhancing tool would be a highly desired and unique approach also for gas fermentation processes, where inorganic gas substances, such as CO, CO_2 and H_2, can be converted into volatile fatty acids (VFAs), alcohols (e.g., ethanol, butanol) or methane (CH_4). Although it should be theoretically beneficial as a biocatalyst, the use of biochar materials for gas fermentation purposes requires extra attention for mass transfer concerns to be compared with existing efficient technologies, such as membrane biofilm reactors (MBfR). To optimise the gas distribution and maximise biofilm colonisation, a biochar-based material with a defined shape and porosity must be produced. Different kinds of char-based composites were previously obtained by combining polyurethane, polypropylene and epoxy [10–12] and lignocellulosic biochar. However, given that available materials do not meet the required features, a new tailored biochar-based composite was developed. The biochar composite casted as a monolith and tested for its homoacetogenic gas fermentation capability in a new type of bioreactor, which is called char-based biofilm sparger reactor (CBSR).

Development of mouldable biochar composite and biochar sparger

PS is one of the most widely used thermoplastics, and Styrofoam is an ubiquitarian waste material. For the lab-scale preparation of the composite, PS is advantageous because it can be softened with a minimal amount of acetone and can be processed at room temperature, thus requiring minimum equipment. Biochar used in this work was previously obtained through slow pyrolysis of lignocellulosic biomass (explained in detail elsewhere [13]). Numerous trial-and-error attempts were conducted to compound biochar and PS successfully and thus obtain a shapable raw material and achieve a rigid but porous monolith in the latter (Fig. 1). During the mock trials for achieving a standardised manufacturing procedure, different strategies, such as changing the biochar/PS ratio and

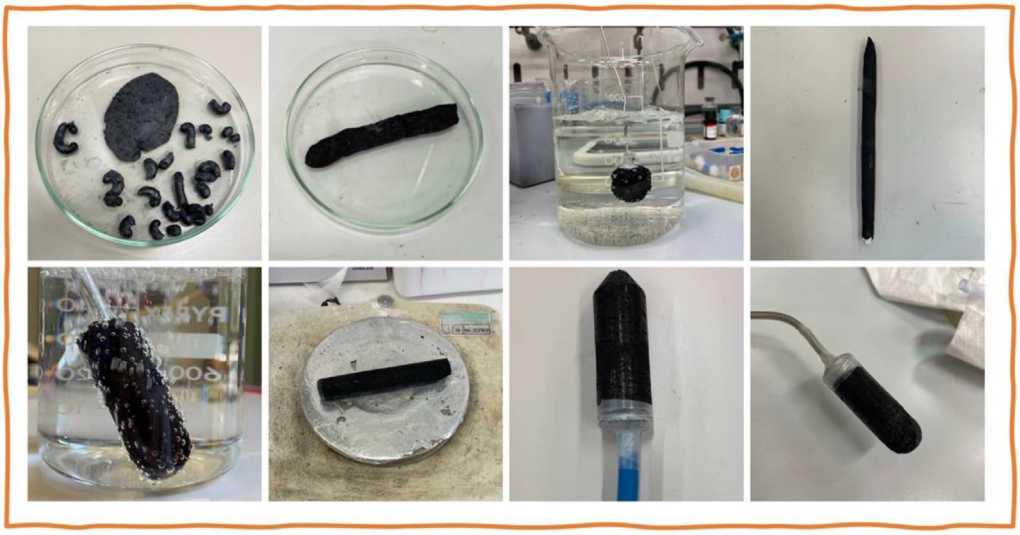

Fig. 1. Some examples of the preliminary mock trials for biochar-made composite monoliths

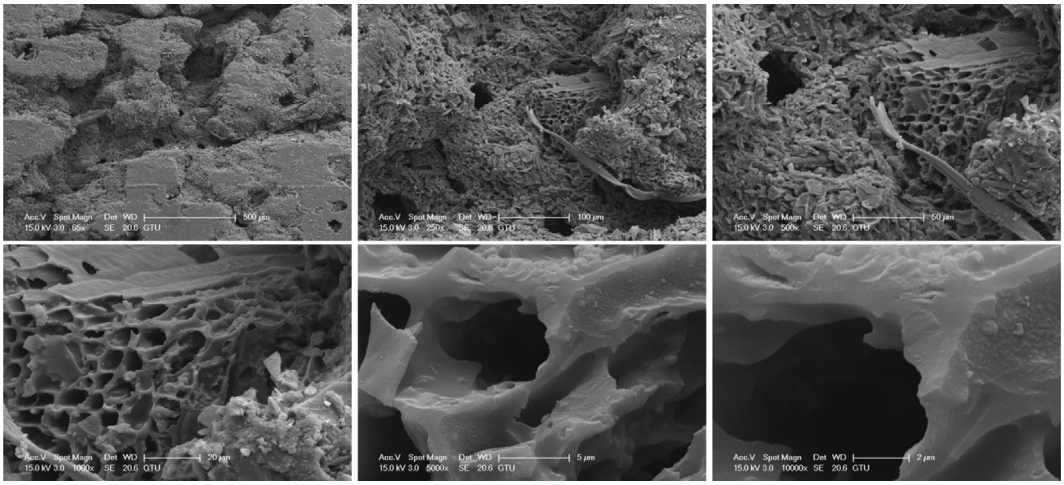

Fig. 2. SEM photographs of the biochar-PS composite material

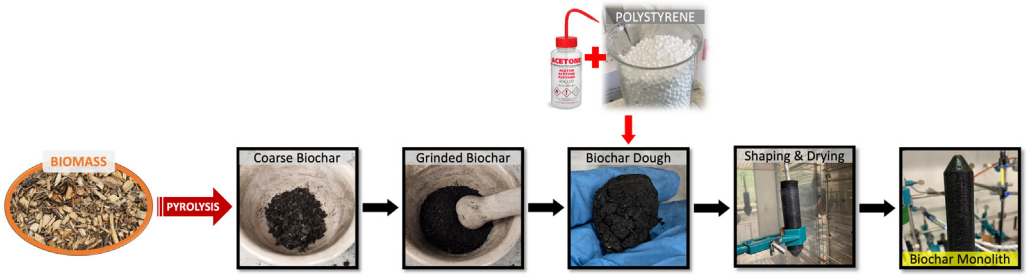

Fig. 3. Manufacturing steps of the biochar–PS composite material to obtain monoliths

Table 1
Geometrical and material characteristics of the spargers used for the test.

Parameters		Commercial Sparger	Biochar Sparger
Material(s)	-	Inert stone	Biochar, Polystyrene
C:H:N:O Ratio[1]	%	n/a	81.0 : 4.2 : 0.6 : 14.3
Biochar : PS Ratio	*M:M*	n/a	2.0
Active Length	*mm*	65.0	65.0
Outer Diameter	*mm*	11.0	14.0 ± 1.50
Inner Diameter	*mm*	8.0	8.5 ± 0.50
Material Volume	cm^3	2.9	6.3
Bulk Density	g/cm^3	0.5	0.8
Porosity	%	68.3	27.5

[1]Carbon, Hydrogen, Nitrogen and Oxygen contents

changing the amount of acetone, compaction ratio, etc., were followed. Eventually, a mouldable char-PS composite was achieved while conserving the biochar's porous structure, as visualised in Fig. 2.

The manufacturing procedure of the biochar-made monoliths are shown in Fig. 3**;** this procedure consists of several steps: grinding of biochar grains (1), screening by a coarse sieve (≈1.0 mm) to obtain a powder with an approximately homogenous particle-size distribution (2), adding acetone-melted PS (≈1–2 ml per g of PS foam) into the powder (3), kneading the mixture of biochar and PS with subsequent additions of acetone to obtain a dough-like material (4), stuffing biochar dough into a pattern to achieve an external shape (5), inserting a rigid pipe to have an internal hole for gas transfer (6), drying in oven at 80 °C for 2 h (7), removing biochar monolith from the external pattern and internal pipe (8) and attaching a plastic hose by glueing with PS to the internal hose of the sparger.

The developed biochar-based porous sparger (***BS***) is submerged into a liquid medium, where the substrate gas is fed. In this manner, gas feeding rate can be regulated by an external regulator or a pump, and the gaseous substrates are forced to perforate through the pores of the ***BS*** where the attached grown microorganisms are expected to be grown. The geometrical details and physicochemical characteristics of the spargers are presented in Table 1.

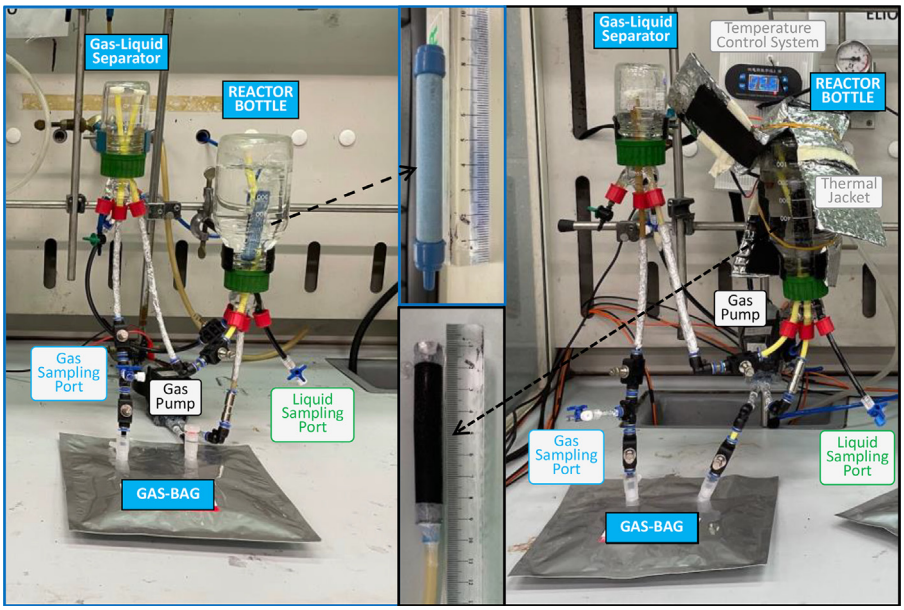

Fig. 4. Preliminary reactor system; Control reactor with the inorganic sparger on the left and BS reactor on the right with the biochar-sparger

Table 2
Setup and operational parameters of the *BS* performance test.

Parameters		Control Reactor	BS Reactor
Sparger Type	-	Commercial	Biochar made
Gas Substrate	-	Hydrogen (H_2)	Hydrogen (H_2)
Operational Method	-	Daily Fed Continuous	Daily Fed Continuous
Temperature Set	*C*	36.0	36.0
Total Wet Volume	*mL*	500	500
Hydraulic Retention Time	*d*	15	15
Average Gas Loading Rate	$g_{COD}\ L^{-1}\ d^{-1}$	0.68 ± 0.26	0.68 ± 0.26
Gas Circulation Flow Rate	*L/min*	1.0	1.0
Circulation Pump Flow Regime	*sec*	2.5 ON / 12.5 OFF	2.5 ON / 12.5 OFF
$NaHCO_3$ Level in Feeding*	*g/L*	40.0	40.0

*Bicarbonate was supplied as a dissolved CO_2 source to maintain an adequate amount of carbon source for the homoacetogenic biochemical reaction [Eq. 1].

Preliminary reactor system

To test the performance of the manufactured ***BS*** material, a tailor-made bioreactor system was developed. For this purpose, a preliminary reactor system was designed to conduct an initial biological gas fermentation test. The full setup consisted of two identical bench-scale gas fermenters with different gas-fixation tools (Fig. 4). One reactor was equipped with the ***BS*** presented in the previous section ('BS reactor' hereinafter), whereas the other was equipped with a commercial inorganic sparger (control reactor) (Table 2).

The bioreactor was made in a standard 500 mL Pyrex bottle, where fermentation occurred, and a secondary smaller Pyrex bottle (100 mL) was used to remove moisture in the exiting gas. The main reactor's bottle was equipped with a special four-port screwcap and placed upside down. The four ports were used for gas injection (1), liquid sampling (2), gas discharging (3) and as a liquid connection port from the condenser bottle (4). The secondary bottle was used as a gas–liquid separator (i.e., gas drain, hydraulic compensator) to minimise the amount of liquid loss due to evaporation and liquid droplets jumping to the gas-discharging hose due to the bubbling. The working principle of the gas–liquid separator was simple but efficient to prevent liquid loss in the long-term continuous operation of the bioreactor setup. The temperature difference between the two bottles was appropriately 15 ˚C–20 ˚C because an external heating system was only applied to the reactor bottle (36±2 ˚C), and the gas–liquid separator was exposed to the laboratory temperature conditions (20±5 ˚C). This difference in temperature conditions between two bottles was enough to sustain an adequate recondensation of the liquid medium. Moreover, the gas–liquid separator was replaced in an upside-down position but in a slightly higher elevation level than the reactor bottle to sustain hydraulic flow towards the reactor bottle. In this manner, condensed liquids were forced to flow back to the reactor where the biological conversion occurs. The gas–liquid separator bottle was also

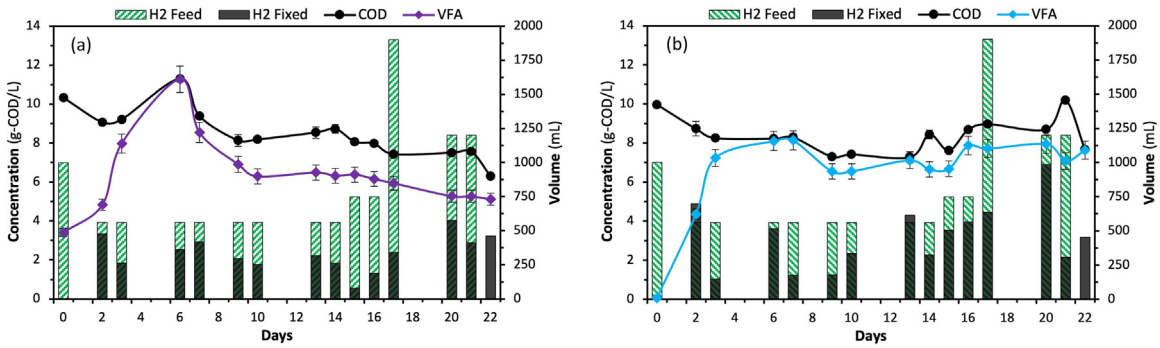

Fig. 5. Profile graphs of H2 feed/fixed, soluble COD and total VFA parameters for the control (a) and BS (b) reactors

combined with a multiport screwcap and the ports were used as gas inlet from the reactor bottle (1), condensed liquid back-drainage to the reactor bottle (2), gas outlet to the gasbag (3) and a reserved liquid port for final discharging (4).

Special attention was given to the gas tightness of the reactor system because H_2 is a highly permeable gas. The reason behind the positioning of the reactor bottles upside down was to provide extra efficiency in terms of leaking. In this manner, all the connection points and ports stay submerged in liquid, remarkably decreasing the chance of any undissolved gas leakages. Additionally, all external plastic (PA12) hoses were laminated by silicone and aluminium with a multilayer approach, similar to previous work [9]. All valves and sample ports were chosen as gas-tight materials. In addition, the vacuum-type mini gas pumps were specifically chosen and have been subjected to numerous tests for gas tightness after isolation through external silicone application into the connection points. Before the gas fermentation experiment, the complete bioreactor setup was filled with helium and hydrogen to evaluate (with three days long leak tests) gas and air leaks.

Biochar-made sparger (*BS*) performance test

To test the performance of the novel ***BS*** material as a biological gas fixation tool against a commercial porous stone sparger with a similar shape, a homoacetogenic gas fermentation [Eq. 1] experiment was conducted. H_2 was used as the sole chemical energy source, and CO_2 was provided in dissolved form ($NaHCO_3^-$) as the only carbon source within the *power-to-material* (i.e., *power-to-x*) perspective.

Prior to the ***BS*** performance test, the bioreactor system with two identical units (Fig. 4) was flushed with an excess amount of He to scavenge the atmospheric air. A known amount (10 mL) of basal medium was supplied to the reactor bottle on the basis of a previous study published elsewhere [8]. A digestate sample obtained from an agricultural industrial wastewater treatment plant (Caviro Extra S.p.A.) was used as microbial mixed culture (MMC) inoculum (≈0.5g as VS). The chemical characteristics and the taxonomical characterisation of the inoculum can be found elsewhere [8]. A small amount of glucose (4.5 g) was added to the system during the startup to sustain a rapid enrichment of MMC throughout the reactor systems. Besides, sodium 2-bromoethanesulfonate (BES) chemical was also provided at 10 mM concentration level to inhibit methanogenic archaea. The initial COD content of both reactors were adjusted to 10 g-COD/L level. All the analytical measurements were conducted according to the methods already detailed in the related research article [14].

$$4H_2 + 2CO_{2(aq)} \rightarrow CH_3COO^- + H^+ + H_2O \quad (1)$$

After the startup, during the second day of operation, all the available glucose was consumed by the microbial consortia. The exhaustion of glucose was identified through silylation and GC-MS as described elsewhere [15]. Later, the only chemical energy source for the available microbial consortia was H_2. On the sixth day of operation, suspended microbial communities were removed by centrifuging (for 15 minutes at 4000 RPM) all available liquid suspensions in the reactors. In this manner, only attached-grown microbial communities, that is, biofilm onto the spargers, were supposed to be the only bioactive area of the system.

VFA content was rapidly increased up to 11.3 g_{COD}/L until the removal of suspended microbes (day 6) in the control reactor, followed by a sudden decrease and a semi steady phase around 6 g_{COD}/L between days 10 and 17 (Fig. 5). Later, the final overall VFA value was approximately 5 g_{COD}/L. The possible reason behind the nonstable and decreasing VFA trend might be correlated with a possible insufficient biofilm formation of the target homoacetogenic microbes onto the commercial sparger. Moreover, a rapid fall in VFA concentration just after the microbial clarifying procedure (centrifuging) at day 6 implied that the suspended consortia were the leading actor of H_2/CO_2 bioconversion for the control reactor with commercial sparger. By contrast, the highest and equal concentrations of COD and VFA were recorded at day 6, when suspended and attached grown microorganisms were biologically active. The commercial sparger had higher porosity with uniformly distributed pores, which created finer bubbles, as compared with the carbonaceous ***BS*** (Table 1). In addition, given the higher material volume of the ***BS***, it was expected to have a more enriched attached-grown microbial community, whereas the commercial one could possibly ensure a better performance due to the possibly better bubbling performance during the initial six-day period when the suspended microbes were not eliminated (Fig. 6).

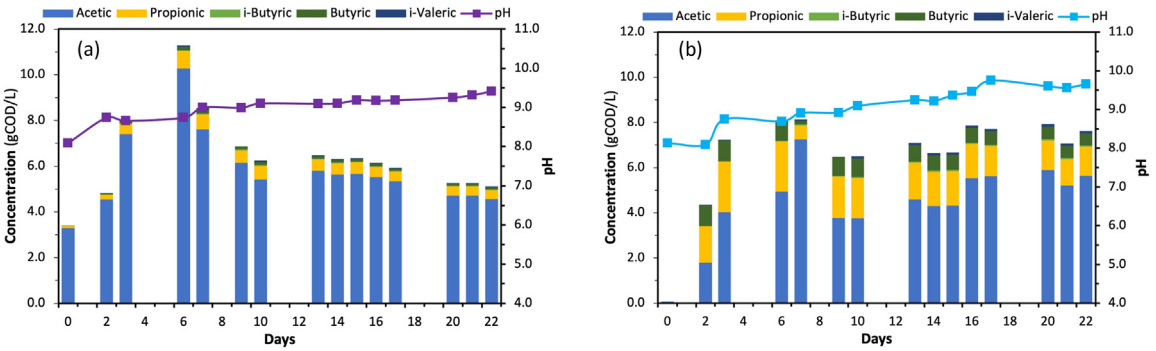

Fig. 6. Profile graphs of VFA composition and pH parameters for the control (a) and BS (b) reactors.

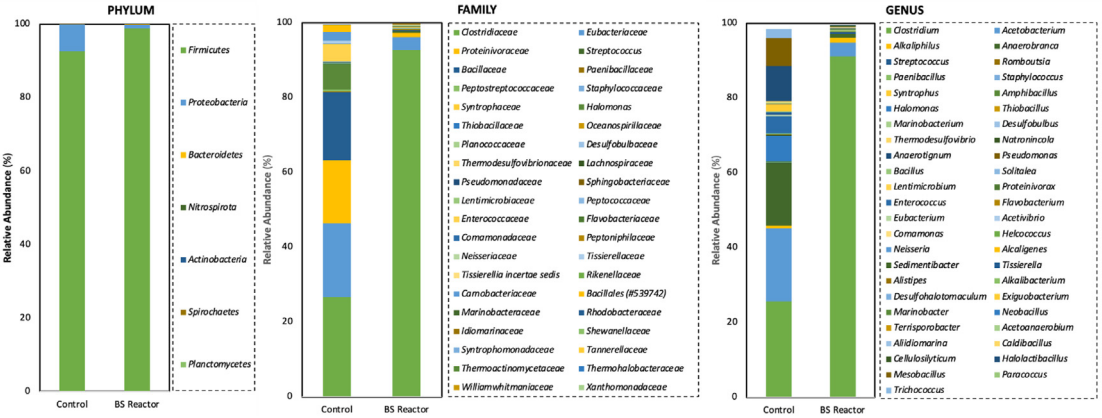

Fig. 7. Most abundant microbial communities found in the performance test of the control and BS reactors.

Table 3
Performance parameters of the test.

Parameters	Unit	Control Reactor	BS Reactor
COD Recovery	%	85%	83%
Gas Fixation Ratio	$\%_{COD}$	42%	57%
VFA_{NET} : Gas_{FIXED} *	$\%_{COD}$	23%	45%
Sparger Productivity *,☆□	$g_{COD}\ L^{-1}\ d^{-1}$	6.7	8.5

* Based on net VFA production originating from the gas substrate (glucose contribution was extracted).
☆□ Unit volume was defined as the sparger's volume as the sole biologically active surface of the system.

In the case of the BS reactor, an initial increasing trend was observed for VFA content, reaching up to the 8 g_{COD}/L overall level. Later, similar to the control reactor, a decline was observed starting on the second week of operation. However, an increase in VFA content occurred, ending with a stable period around the 8 g_{COD}/L level, in contrast to the control reactor. As can be tracked from Fig. 5, the BS reactor showed better daily H_2 uptakes for most of the period, resulting in higher soluble COD and total VFA concentrations in comparison with the control reactor equipped with a commercial standard sparger. By contrast, pH trends showed a comparable continuous increasing trend, resulting in approximately pH 9.5 for both cases. Acetic acid was the most dominant VFA type for both cases, and another remarkable difference in the results was defined for the VFA compositions. Acetic acid proportions for the last three-day stable period of the test were found as 89% and 67% for the control and BS reactors, respectively. The rest mainly consisted of propionic and butyric acids for the BS reactor, i.e., 22% and 9% of overall VFA content, respectively.

An overall performance summary of the preliminary biological performance test is presented in Table 3 via indicative parameters. Both closed-loop bioreactors ended up with a COD balance above 83%. By taking account of some of the input COD should have been captured as microbial biofilm growth, such COD balance can be considered satisfactory. The BS reactor provided a higher gas fixation ratio, VFA yield (e.g., VFA_{NET} : Gas_{FIXED}) and sparger productivity than the control reactor. Results confirmed that gas fermentation

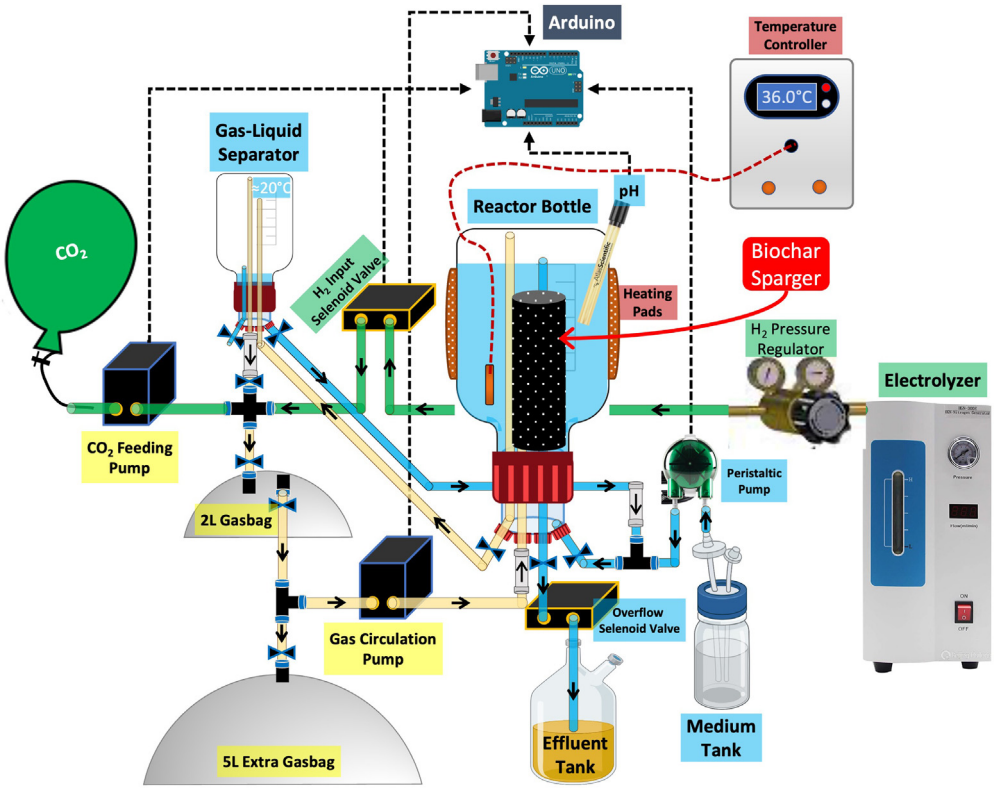

Fig. 8. Methodological scheme of the final CBSR setup and connected apparatus.

can occur by using a microbial biofilm attached onto the sparger, suggesting that the material /structure of the sparger can be relevant for the fermentation performance.

To investigate the presence of microbial communities, the spargers were ground and subjected to 16S rRNA sequencing. Fig. 7 shows the taxonomic composition, revealing that the bacterial communities, which become completely different from the inoculum [8], change in a different way according to the support material. For instance, *Clostridium* was found to be the most dominant genus in both cases, i.e., 91% of the overall microbial consortia for the BS reactor and 26% for the control. *Clostridium* was followed by *Acetobacterium* with a 20% and 4% dominancy for the BS and control reactors, respectively. These two most dominant strains can be considered the target strains because they are both under the category of homoacetogens.

Char-based biofilm sparger reactor (CBSR) setup

On the basis of the performance test, which was conducted in a demo setup with a basic apparatus, a new complete bioreactor system was designed to conduct gas fermentation research with the biochar-made sparger ('CBSR' hereinafter) (Fig. 8). This novel CBSR system was developed on the basis of the biochar-packed anaerobic bioreactor, which was previously manufactured, tested and published in detail elsewhere [9]. Similar to the previous bioreactor system, CBSR was supported by the Arduino integrated development environment (IDE) which can also be called a mini-PLC system.

CBSR consists of different units: a reactor bottle (500 mL Pyrex bottle); a gas–liquid separator (100 mL Pyrex bottle); two gas bags connected to each other in parallel (2 and 5 L multilayer foil sampling bag); a water electrolyser as H_2 generator; a 10 L gas bag manually filled with pure CO_2; several add-on units, such as pumps, valves, pH electrodes, etc. All the electronic units, such as pumps, pH electrodes and valves, were controlled by the Arduino board, as shown in Fig. 8 with dashed lines. A more detailed schema of the electrical connections can be found in in Supplementary material.

The reactor bottle was used upside down and equipped with a four-port cap (submerged for minimising gas leaking) and external heating pads. A newer and bigger biochar-made sparger was placed inside the reactor bottle as the biological gas fixation tool. Liquid input and output were provided through the bottom part of the bioreactor via one peristaltic feeding pump and a solenoid valve for discharging overflows. One mini gas pump (i.e., vacuum pump) was used to circulate the headspace gas, which is directly communicated with the external gasbags.

CBSR was designed to be operated in continuous mode operation in terms of feeding and discharging of both gas and liquid materials. However, the number of input (liquid medium, H_2 and CO_2) and output (fermented liquid) subsequent operational cycles can be adjusted by the operator via the developed Arduino sketch (full sketch can be found in in Supplementary material.). In brief,

Arduino was used to develop a sketch in which a cycle of four hours occurs (six times per day). Through a relay module and MOSPHET components, Arduino delivers electricity to pumps and valves. The gas pump was tuned to work in pulse–width modulation (PWM), namely with the possibility to regulate the gas flow from the sketch. Additionally, it can be adjusted to work in consecutive on/off cycles to achieve pulse-mode gas recycling to enhance the biofilm formation onto the ***BS***. Each cycle is composed of a main part, in which only gas recirculation and pH monitoring occur and a feeding-withdrawal cycle (FWD) at the end of the fourth hour. FWD is formed by a sequence of injection and withdrawal of fluids. Injected and withdrawn fluid amounts at each cycle correspond to one-sixth of the daily need. Such system provides to the user with the possibility of editing main operational parameters, allowing high-customizability of the reactor. However, for this setup, a gas discharging pump was not included because it was supposed that the biological reaction can consume all the input gas and convert it into water-soluble products.

Summary

In this study, a new type of biological gas fixation tool that is made by a biochar composite material was developed following numerous preliminary trial and error, during which several different manufacturing strategies including the use of different material ratios, or drying conditions, were tested. In the end, a final manufacturing procedure was achieved for casting the biochar composite as a stiff monolith in a predetermined shape while preserving the porous structure of biochar. Later, in a preliminary semi-continuous bioreactor system, biochar-sparger named monolith, was tested for its biological gas conversion ability against a control reactor that was equipped with a commercial stone-sparger. The test was suggesting that the biochar-sparger had an unquestionable advantage over the control in terms of end product concentration (8 gCOD/L), VFA productivity (8.5 gCOD L^{-1} d^{-1}), and biological gas fixation ability (36% times more than control). Furthermore, microbial genome sequencing demonstrated that the BS reactor also procured a better selection of target bacteria such as *Clostridium*. Following this, a brand-new, fully continuous, advanced bioreactor system with a ten times larger biochar-sparger was constructed and named as “char-based biofilm sparger reactor” in order to carry out a long-term gas fermentation research study for revealing the limits of the discovered tool. While the improved results and great achievements obtained by the advanced CBSR system is the subject of the related research article [14].

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yusuf Küçükağa: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration, Funding acquisition. **Andrea Facchin:** Conceptualization, Methodology, Software, Writing – review & editing, Visualization. **Aaron Alfonsi:** Methodology, Investigation. **Federica Costantini:** Formal analysis, Resources, Investigation. **Serdar Kara:** Writing – review & editing, Supervision. **Cristian Torri:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Data availability

The data that has been used is confidential.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102296.

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